

Application No.: 09/727,855
Amendment Dated: July 19, 2004
Reply to Office Action of: April 21, 2004

AMENDMENTS TO THE CLAIMS

Please amend the claims as follows:

LISTING OF CLAIMS:

Claim 1. (Currently amended) A process for producing carotenoids comprising cultivating in a culture medium a recombinant carotenoid producing organism containing at least one polynucleotide encoding an a-gene for one or more active oxygen species-quenching factor that is disrupted with a disruption cassette specific to the polynucleotide gene, wherein the active oxygen species-quenching factor is encoded by a polynucleotide selected from the group consisting of:

- (a) SEQ ID NOs: 1 or 4, or polynucleotides that hybridize to the complement of SEQ ID NO s: 1 or 4 under high stringency hybridization and wash conditions wherein the hybridizing polynucleotides encode a polypeptide having mitochondrial superoxide dismutase (SOD) activity;
- (b) SEQ ID NOs: 2 or 6, or polynucleotides that hybridize to the complement of SEQ ID NO s: 2 or 6 under high stringency hybridization and wash conditions, wherein the hybridizing polynucleotides encode a polypeptide having cytoplasmic superoxide dismutase (SOD) activity; and

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(c) SEQ ID NOs: 3 or 8, or polynucleotides that hybridize to the complement of SEQ ID NOs: 3 or 8 under high stringency hybridization and wash conditions, wherein the hybridizing polynucleotides encode a polypeptide having catalase activity; and
recovering carotenoids from the culture.

Claim 2. (Original) A process according to claim 1 wherein the recombinant organism belongs to the kingdom of *Monera, Protista or Fungi*.

Claim 3. (Original) A process according to claim 1 wherein the recombinant organism belongs to a genus selected from the group consisting of *Erwinia, Rhodobacter, Myxococcus, Flavobacter, Paracoccus, Synechococcus, Synechocystis, Agrobacterium, Streptomyces, Haematococcus, Dunaliella, Phaffia, Xanthophyllomyces, Neurospora, Rhodotorula, Blakeslea, and Phycomyces*.

Claim 4. (Original) A process according to claim 3 wherein the recombinant organism is a strain of *P. rhodozyma*.

Claim 5. (Original) A process according to claim 4 wherein the recombinant organism is *P. rhodozyma* ATCC 96594.

Claim 6. (Cancelled).

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Claim 7. (Currently amended) A process according to claim 1 wherein the active oxygen species-quenching factor is factors are encoded by a ~~polynucleotide selected from the group consisting of SEQ ID NOs: SEQ ID NO: 1, 2, 3, 4, 6 and 7.~~

Claim 8. (Withdrawn) A recombinant organism for producing carotenoids comprising a gene for at least one active oxygen species-quenching factor, which gene is substantially disrupted with a disruption cassette specific to the gene.

Claim 9. (Withdrawn) A recombinant organism according to claim 8 wherein the recombinant organism belongs to the kingdom of *Monera, Protista or Fungi*.

Claim 10. (Withdrawn) A recombinant organism according to claim 9 wherein the recombinant organism belongs to a genus selected from the group consisting of *Erwinia, Rhodobacter, Myxococcus, Flavobacter, Paracoccus, Synechococcus, Synechocystis, Agrobacterium, Streptomyces, Haematococcus, Dunaliella, Phaffia, Xanthophyllomyces, Neurospora, Rhodotorula, Blakeslea, and Phycomyces*.

Claim 11. (Withdrawn) A recombinant organism according to claim 8 wherein the active oxygen species-quenching factor to be disrupted is selected from the group consisting of mitochondrial superoxide dismutase (SOD), cytoplasmic superoxide dismutase (SOD), catalase, and combinations thereof.

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Claim 12. (Withdrawn) A disruption cassette for disrupting a gene coding for an active oxygen species-quenching factor effective in carotenogenesis in a carotenogenic organism comprising a nucleotide sequence that codes for an active oxygen species-quenching factor that is substantially identical to a part of a DNA sequence coding for an active oxygen species-quenching factor and a selectable marker gene.

Claim 13. (Withdrawn) A disruption cassette according to claim 12 wherein the organism belongs to the kingdom of *Monera, Protista or Fungi*.

Claim 14. (Withdrawn) A disruption cassette according to claim 13 wherein the organism belongs to a genus selected from the group consisting of *Erwinia, Rhodobacter, Myxococcus, Flavobacter, Paracoccus, Synechococcus, Synechocystis, Agrobacterium, Streptomyces, Haematococcus, Dunaliella, Phaffia, Xanthophyllomyces, Neurospora, Rhodotorula, Blakeslea, and Phycomyces*.

Claim 15. (Withdrawn) A disruption cassette according to claim 12 wherein the active oxygen species-quenching factor to be disrupted is selected from the group consisting of mitochondrial superoxide dismutase (SOD), cytoplasmic superoxide dismutase (SOD), catalase, and combinations thereof.

Claim 16. (Withdrawn) A disruption cassette according to claim 12 wherein the nucleotide sequence coding for an active oxygen species-quenching factor is identical

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to at least a part of a polynucleotide sequence coding for the active oxygen species-quenching factor of the organism into which the disruption cassette is to be introduced.

Claim 17. (Withdrawn) A disruption cassette according to claim 16 wherein the nucleotide sequence that codes for an active oxygen species-quenching factor, and that is identical to a part of the polynucleotide coding for an active oxygen species-quenching factor comprises a deletion and/or mutation compared to the corresponding functional gene.

Claim 18. (Withdrawn) A recombinant DNA sequence coding for an active oxygen species-quenching factor effective in carotenogenesis in a carotenogenic organism.

Claim 19. (Withdrawn) A recombinant DNA sequence according to claim 18, wherein the organism belongs to the kingdom of *Monera*, *Protista* or *Fungi*.

Claim 20. (Withdrawn) A recombinant DNA sequence according to claim 19 wherein the organism belongs to the kingdom of *Monera*, *Protista* or *Fungi*.

Claim 21. (Withdrawn) A recombinant DNA sequence according to claim 20 wherein the organism belongs to a genus selected from the group consisting of *Erwinia*, *Rhodobacter*, *Myxococcus*, *Flavobacter*, *Paracoccus*, *Synechococcus*, *Synechocystis*,

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Agrobacterium, Streptomyces, Haematococcus, Dunaliella, Phaffia, Xanthophyllomyces Neurospora, Rhodotorula, Blakeslea, and Phycomyces.

Claim 22. (Withdrawn) A recombinant DNA sequence according to claim 18 wherein the recombinant DNA sequence is isolated from a microorganism of *P. rhodozyma*.

Claim 23. (Withdrawn) A recombinant DNA sequence according to claim 22 wherein the microorganism is *P. rhodozyma* ATCC 96594.

Claim 24. (Withdrawn) A recombinant DNA sequence according to claim 18 wherein the active oxygen species-quenching factor is a mitochondrial superoxide dismutase.

Claim 25. (Withdrawn) A recombinant DNA sequence according to claim 24 wherein the mitochondrial superoxide dismutase is encoded by a polynucleotide sequence identified by SEQ ID NO: 1 or 4.

Claim 26. (Withdrawn) A recombinant DNA sequence according to claim 25 wherein the mitochondrial superoxide dismutase is encoded by a polynucleotide sequence that binds under high stringency conditions to the sequence of SEQ ID NO: 1 or 4, and has a mitochondrial superoxide dismutase activity.

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Claim 27. (Withdrawn) A recombinant DNA sequence according to claim 18 wherein the active oxygen species-quenching factor is cytoplasmic superoxide dismutase.

Claim 28. (Withdrawn) A recombinant DNA sequence according to claim 27 wherein the cytoplasmic superoxide dismutase is encoded by a polynucleotide sequence identified by SEQ ID NO: 2 or 6.

Claim 29. (Withdrawn) A recombinant DNA sequence according to claim 28 wherein the cytoplasmic superoxide dismutase is encoded by a polynucleotide sequence that binds under high stringency conditions to the sequence of SEQ ID NO: 2 or 6, and has a cytoplasmic superoxide dismutase activity.

Claim 30. (Withdrawn) A recombinant DNA sequence according to claim 18 wherein the active oxygen species-quenching factor is catalase.

Claim 31. (Withdrawn) A recombinant DNA sequence according to claim 30 wherein the catalase is encoded by a polynucleotide sequence identified by SEQ ID NO: 3 or 8.

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Claim 32. (Withdrawn) A recombinant DNA sequence according to claim 31 wherein the catalase is encoded by a polynucleotide sequence that binds under high stringency conditions to the sequence of SEQ ID NO: 3 or 8, and has catalase activity.

Claim 33. (Withdrawn) A recombinant DNA fragment comprising a coding region for a transit peptide upstream of the coding region of an objective protein.

Claim 34. (Withdrawn) A recombinant DNA fragment according to claim 33 wherein the objective protein is mitochondrial superoxide dismutase.

Claim 35. (Withdrawn) A method for locating an objective protein in mitochondria comprising expressing the recombinant DNA fragment of claim 24 or 25 in a recombinant host organism.

Claim 36. (Withdrawn) A method for cloning a gene encoding an active oxygen species-quenching factor effective in carotenogenesis in a carotenogenic organism comprising providing as a probe or primer a polynucleotide sequence encoding a polypeptide having the activity of a mitochondrial superoxide dismutase (SOD), a cytoplasmic superoxide dismutase (SOD) and/or a catalase.

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Claim 37. (Withdrawn) A method according to claim 36 wherein the polynucleotide sequence is selected from the group consisting of SEQ ID Nos:1, 2, 3, 4, 6, 8, and fragments thereof.

Claim 38. (New) A process according to claim 1 wherein the active oxygen species-quenching factor is encoded by a polynucleotide selected from the group consisting of polynucleotides that hybridize to the complement of SEQ ID NO: 1 or SEQ ID NO: 4 under high stringency hybridization and wash conditions wherein the hybridizing polynucleotides encode a polypeptide having mitochondrial SOD activity.

Claim 39. (New) A process according to claim 38 wherein the active oxygen species-quenching factor is encoded by a polynucleotide sequence comprising SEQ ID NO: 1 or SEQ ID NO: 4.

Claim 40. (New) A process according to claim 1 wherein the active oxygen species-quenching factor is encoded by a polynucleotide selected from the group consisting of polynucleotides that hybridize to the complement of SEQ ID NO: 2 or SEQ ID NO: 6 under high stringency hybridization and wash conditions wherein the hybridizing polynucleotides encode a polypeptide having cytoplasmic SOD activity.

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Claim 41. (New) A process according to claim 40 wherein the active oxygen species-quenching factor is encoded by a polynucleotide sequence comprising SEQ ID NO: 2 or SEQ ID NO: 6.

Claim 42. (New) A process according to claim 1 wherein the active oxygen species-quenching factor is encoded by a polynucleotide selected from the group consisting of polynucleotides that hybridize to the complement of SEQ ID NO: 3 or SEQ ID NO: 8 under high stringency hybridization and wash conditions wherein the hybridizing polynucleotides encode a polypeptide having catalase activity.

Claim 43. (New) A process according to claim 42 wherein the active oxygen species-quenching factor is encoded by a polynucleotide sequence comprising SEQ ID NO: 3 or SEQ ID NO: 8.